GAMETOGENESIS AND EARLY DEVELOPMENT OF THE POLYCHAETE GLYCERA DIBRANCHIATA [1]

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A previous paper (Simpson, 1962) has described fall swarming of the bloodworm, *Glycera dibranchiata* Ehlers, observed at Solomons, Maryland, during a study conducted there from late June, 1960, to early February, 1961. The same paper also discussed the probable breeding seasons of this species at Solomons, and presented data on the rate of gonad development, concluding that gametogenesis very likely requires close to a year for completion. A more detailed account of gametogenesis and an outline of development to the trochophore stage, obtained in the course of the same investigation, form the subject of the present report.

An early account of glycerid reproductive organs appears in Ehlers' (1864–68) original description of *G. dibranchiata*, and although inaccurate, has remained the only source of information on gonads in the genus. The embryology of *Glycera* is but slightly better known: Allen (1957) gives some data on the rate of early development in *G. ?sphyrabrancha*, Fuchs (1911) describes the young larval stages of *G. convoluta*, and observations on *G. dibranchiata* from fertilization to the trochophore are reported by Klawe and Dickie (1957). In the last two cases, as in the present one, attempts to maintain cultures beyond the trochophore stage were unsuccessful. This difficulty has also been alluded to by Wilson (1948), and it appears that further concentrated effort will be required to determine the complete developmental history of these annelids.

Gametogenesis

Methods

The following observations are based largely on histological examination of about 70 specimens collected at Solomons (38°19′N., 76°27′W.) between June, 1960, and February, 1961. Animals were narcotized in magnesium chloride, fixed in Bouin's fluid and preserved in a mixture of 2 parts ethanol, 1 part distilled water and 1 part glycerine. Paraffin sections 4–7 μ thick were stained either with Ehrlich's hematoxylin and eosin, or by a modified procedure using Gomori's chrome-alum hematoxylin, recommended as a chromosome stain by Melander and Wingstrand (1953). Schiff's reagent, prepared by several different methods, consistently gave either weak

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or negative Feulgen reactions, and this technique was discontinued. Some attempt was made to study in more detail the chromosomal changes associated with gametogenesis by making squash preparations of gonads; results, however, were entirely unsatisfactory, probably because the material had been fixed and stored for several months. Gonads of fresh worms purchased at a bait store gave promising squash preparations with La Cour's (1941) aceto-orcein method, but this phase of the study was not pursued further.

Gonads

Glycera dibranchiata is dioecious, with segmentally paired gonads beginning in the region of segments 45–47 and extending posteriorly for approximately 60 seg-

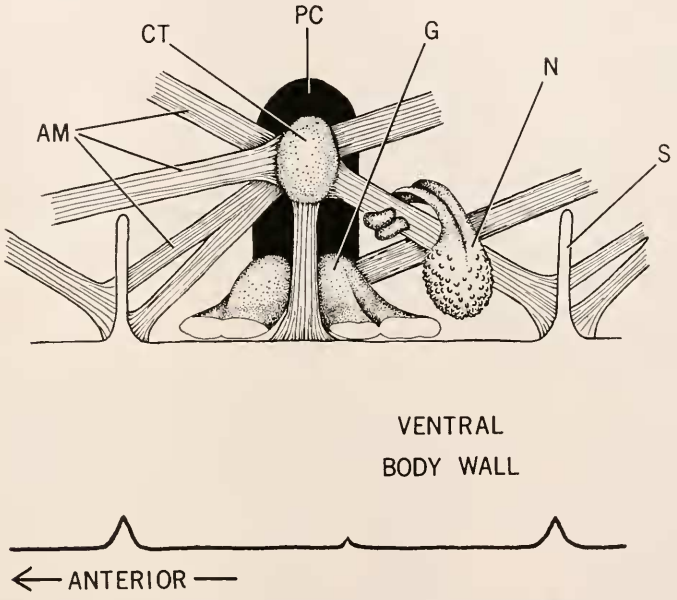

FIGURE 1. Diagram of the ventral half of one body segment, viewed from the median plane. Not to scale. AM, acicular muscles; CT, connective tissue pad around bases of the acicula; G, lobe of gonad; N, nephridial complex; PC, parapodial cavity; S, septum.

ments. Each gonad arises as an outgrowth from connective tissue at the lateral edge of the ventral longitudinal muscles, where the parapodial cavity opens into the general coelom. Between the gonad and the longitudinal muscles lies the origin of the ventral acicular muscle (Fig. 2), which passes dorso-medially from this point and inserts on a connective tissue pad surrounding the bases of the two parapodial acicula. From the same connective tissue pad other acicular muscles also radiate to their various origins on the body wall (Fig. 1); thus, the space into which the gonad grows is limited dorsally by the acicula, and anteriorly, posteriorly and medially by the acicular muscle bands.

The initial gonial swelling gradually enlarges, extending further toward the coelom and occasionally a short distance into the parapodial cavity as well, but

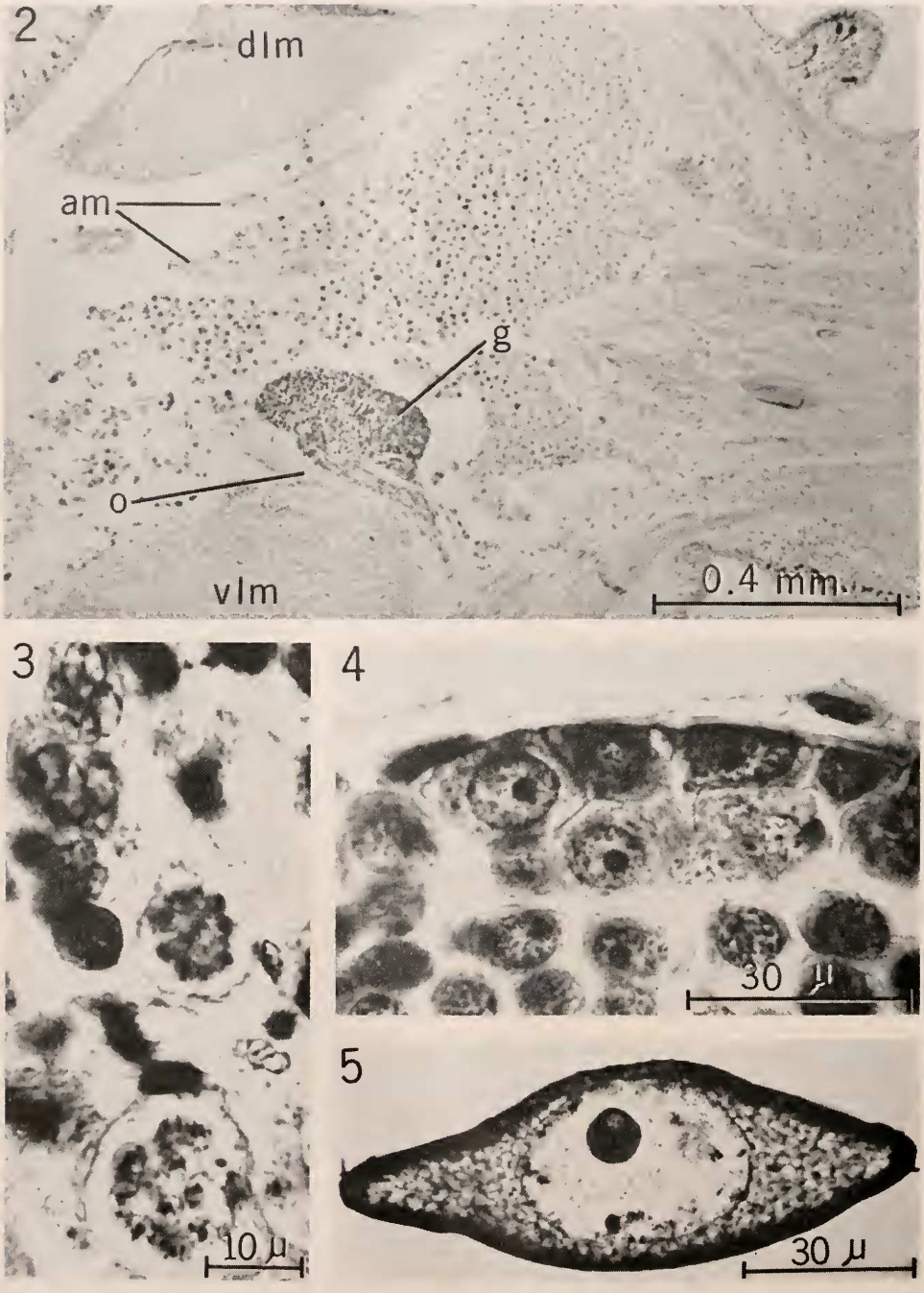

FIGURE 2. Transverse section through a mid-body segment, showing the position of the gonad (g). Part of the intestine appears in the upper left corner, and the dorsal cirrus of the parapod in the upper right corner. am, acicular muscles; dlm and vlm, dorsal and ventral

remains attached to its point of origin by a broad, compact stalk, which penetrates into the underlying connective tissue (Fig. 2). The nephridial duct from the preceding segment usually passes near or through this region of the gonad before turning laterally to the small nephridial pore just below the parapod. As the gonad expands toward the coelom, it becomes dorso-ventrally flattened between the acicula and their muscles, and eventually it pushes out between these muscle bands to form irregular lobes projecting into the body cavity. Since the degree of lobulation attained is subject to individual variation, no average dimensions can be indicated; maximum cross-sections range from 70 × 55 μ for very small gonads to 420 × 360 μ for large ones.

In dissections, large gonads can at times be confused with the nephridial complexes, which are also segmentally paired. The latter organs have the same general structure as those described in other *Glycera* species (Goodrich, 1898; Fage, 1906), but instead of being attached to the anterior face of the septum, each nephridial complex is connected only to the posterior acicular muscle (Fig. 1). The distal end of the ciliated organ makes a turn around this muscle, and the nephridial duct continues down the muscle to the body wall. Thus the main portion of the nephridial complex, consisting of the phagocytal sac and the protonephridium with its solenocytes, projects freely into the coelom and is occasionally found lying between the acicular muscles in the region of the gonad. In live specimens, the nephridial complex has a yellow-green tint readily distinguishable from the light pink of the reproductive organs.

The gonads of *Glycera* have previously been described by Ehlers (1864–68), but his account is somewhat confusing, since it is not entirely clear whether the description pertains wholly to *G. dibranchiata* or also includes *G. capitata*. His description of the ovaries (pp. 697–700) seems to be based on examination of epitokous females of *G. capitata*, yet the figures referred to are all labeled *G. dibranchiata*. These structures certainly bear little resemblance to the gonads described here; neither the "grape-like clusters" nor the simpler, fiber-like ovaries that Ehlers described have ever been observed in the present study. Besides, the females examined by Ehlers must have been far advanced in sexual maturity (as indicated by their pronounced atrophy and the masses of eggs in their body cavities), and according to the present observations, these specimens should have had no remaining gonad tissue whatsoever. On the other hand, the description of what Ehlers believed to be the testes (p. 700) is explicitly based on a specimen of *G. dibranchiata* and does agree with the present findings.

The matter is further complicated by Lubischev (1924), who states that Ehlers' illustrations of ovaries represent exactly the multiple nephridial complexes found in *G. capitata*. Since these illustrations are indicated as *G. dibranchiata*, Lubischev

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longitudinal muscles; o, origin of ventral acicular muscle. Melander-Wingstrand's modified chrome-alum hematoxylin (CAH).

FIGURE 3. Gonadal cells with basophilic material enclosed in separate vesicles (bottom and center of figure). Two cells at the upper left show the basophilic substance distributed around the periphery of each vesicle. Melander-Wingstrand's CAH.

FIGURE 4. Young oocytes at the surface of the gonad, shortly before they are released into the coelom. Gomori's CAH, phloxin.

FIGURE 5. Section through an oocyte shed spontaneously in the laboratory. Ehrlich's hematoxylin, eosin.

concludes that similar multiple nephridia are present in this species also, and that Ehlers must have mistaken them for ovaries. This conclusion, however, is incorrect, for *G. dibranchiata* has only two nephridial complexes in each segment, and these organs distinctly differ from the structures Lubischev found in *G. capitata*. It appears therefore that the "ovaries" described by Ehlers were in fact nephridial complexes of *G. capitata*, the legends to his figures notwithstanding. His misinterpretation was undoubtedly due to the presence of eggs in these structures, but it is not at all unusual to find gametes in the sac of the ciliated organ, which is closely associated with the nephridium proper (see Goodrich, 1945). Ehlers' description of the segmental organ, although not entirely accurate, indicates that he did recognize the nephridial complex in *G. dibranchiata*.

Early gametogenesis

The gonad first appears as an aggregation of cells embedded in connective tissue near the base of each parapod. The origin of these cells is uncertain, but they resemble the small, apparently amoeboid, basophilic cells scattered throughout connective and muscular tissues. The clustered gonial cells enlarge and proliferate upward, producing a bulge beneath the coelomic epithelium, which forms an investing membrane around the projection. From this early stage onward, the gonad is marked by the presence of relatively large (about $20 \times 11\ \mu$) oval cells containing scattered concentrations of chromatin. These cells are somewhat localized toward the interior of the gametal mass and in larger gonads occasionally seem to form a core extending from the stalk into the central region of the organ. There is, however, no sharp division into cortical and medullary zones, and the cells may occur in other parts of the gonad as well.

The chromatin inclusions within the cell vary in size and density, larger ones generally being more diffuse, with indistinct outlines, and smaller ones more compact and well defined. Their basophilia also varies with the degree of concentration, but never becomes very intense. In some cases, each inclusion appears to lie in a separate vesicle, giving the distinct impression that the larger body consists of a number of minute cells (Fig. 3). From this condition there follows a series of stages in which the basophilic material is distributed around the inner surface of each compartment, the individual vesicles coalesce, and the whole structure takes on the appearance of a cell with a large nucleus surrounded by a thin layer of cytoplasm. The significance of these cells in the gametogenetic process is not clear. In some aspects they strongly resemble stages of orthopteran spermatogonial divisions, in which individual chromosomes are separately compartmentalized (*e.g.* Wenrich, 1916; Rao, 1934), and it is possible that a similar situation occurs in *Glycera*.

The bulk of the gonad consists of developing gametes, which are at first closely packed and of uniform appearance, each cell containing a large reticulated nucleus surrounded by a small amount of cytoplasm. After a certain period, during which the gonad continues to grow, the germ cells enter a phase of nuclear activity and become more or less segregated into small groups, each containing cells at a similar stage of development. Although metaphase and anaphase configurations are relatively scarce, the nuclear chromatin shows various prophasic changes, undergoing condensation into thread-like filaments that thicken, become more basophilic, and

form a compact tangled knot. Chromosome counts indicate that the diploid number probably lies between 30 and 40. Up to this point, ovaries and testes cannot be distinguished.

Oogenesis

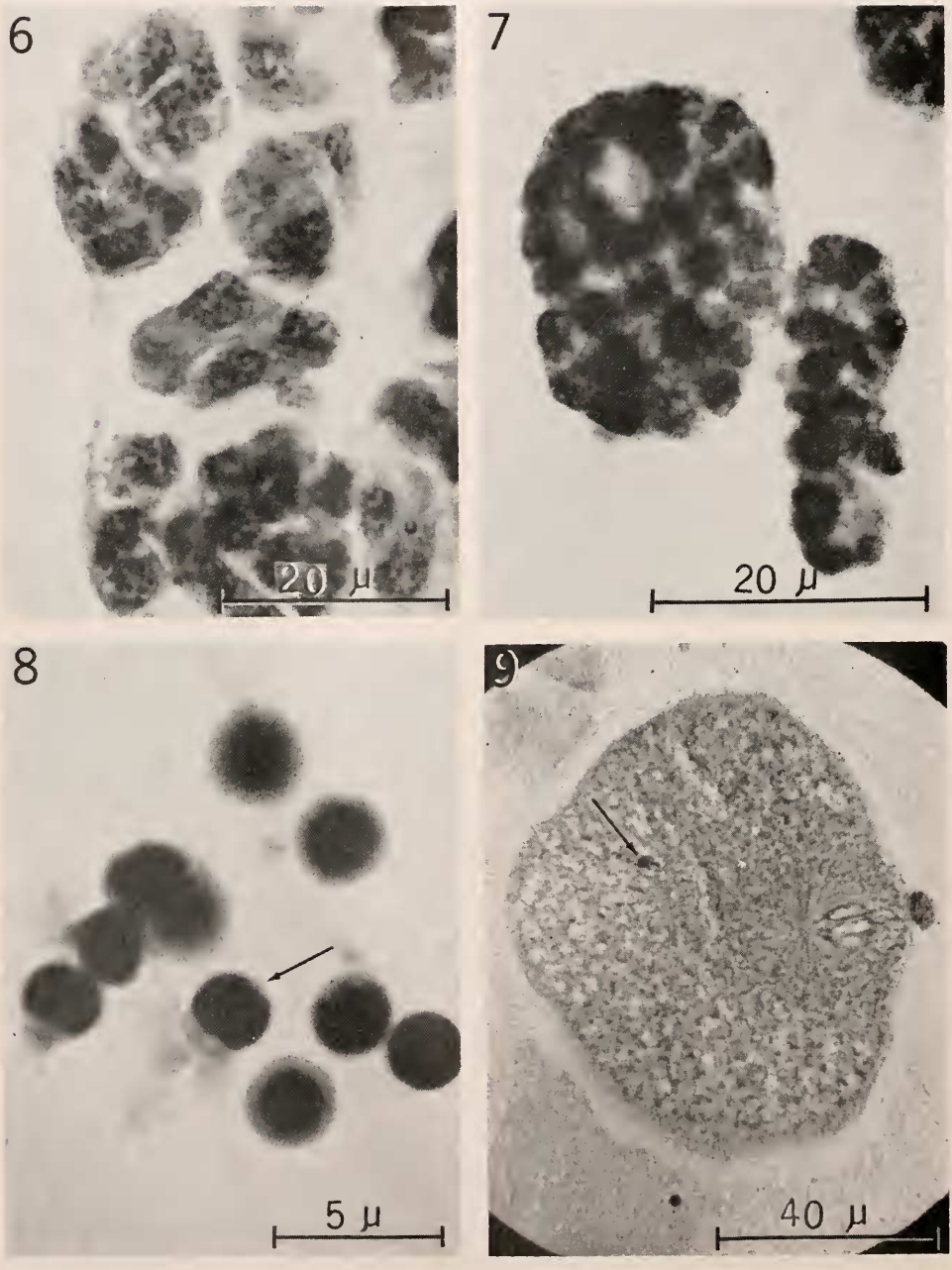
The first definite sign of sexual difference is the appearance of oocytes in the peripheral layers of the gonad, especially along its medial border. In these cells the dense chromatin mass relaxes into a diffuse network, a nucleolus appears, and the cytoplasmic volume begins to increase (Fig. 4). The stage at which the oocytes leave the gonad seems to vary. Apparently they are ready to be released upon reaching a diameter of about 28 μ, but occasionally the rupture of the epithelial covering is delayed, and they may grow larger before escaping into the coelom. As the oocytes are progressively released, the ovary decreases in size until no sign of it remains in gravid females. The oocytes complete their growth while circulating in the coelomic fluid, attaining an average diameter of 140 μ before being shed. No marked nuclear changes take place during this period, and since both polar bodies are formed after fertilization, it appears that the egg chromosomes rest in a diffuse state, having passed through early prophase of meiosis I in the gonad.

The ripe oocyte (Fig. 5) is a flattened sphere, bulging over the centrally placed germinal vesicle and surrounded by a thin membrane. Discoidal eggs have been described for other *Glycera* species (Fuchs, 1911; Allen, 1957) and are probably characteristic for the whole family. The colorless cytoplasm of the living egg is granular, with some spherical, refractile inclusions that may be small oil droplets; a thin cortical zone of fine granules is present. Oocytes fixed in Bouin's show a basophilic, alveolar cytoplasm. The germinal vesicle measures 50 μ in diameter and contains a diffuse acidophilic reticulum. The nucleolus is about 14 μ in diameter and appears to be double, consisting of a weakly basophilic, vacuolated portion that is cupped around a more homogeneous, acidophilic center.

Spermatogenesis

The spermatocytes similarly appear first in the periphery of the gonad, where they occur in small clusters of seven or eight cells (Fig. 6), which remain together after being released into the coelom. As in the female, the male gonad also becomes smaller and eventually disappears. When first released, individual spermatocytes have a diameter of 6 μ and contain a large nucleus with chromatin evenly distributed in the form of darkly staining condensations. The spermatocyte clusters develop into oval plates several cells thick, each plate consisting of approximately 30 or 40 cells and measuring about 16×9 μ (Fig. 7). Although division figures have not been observed, it seems likely that this increase in cell number is due to the maturation divisions. The nucleus of each spermatid is a rounded mass of chromatin, which appears to be honeycombed with minute vacuoles. The cytoplasm is reduced to a very thin layer and soon disappears entirely. With increasing condensation of the chromatin, the spermatids assume a somewhat triangular shape, while the sperm plates begin to loosen and break up. Apparently, spermiogenesis is completed after the individual spermatids have separated.

The mature spermatozoon (Fig. 8) is of the primitive type. Its spherical



FIGURES 6–9.

head is 2.5 μ across, bears a rounded acrosome, and is slightly flattened at the posterior border, where the four round elements of the middle piece are arranged around the base of the flagellum. Sperm remain active for at least six hours after being shed, but it is not known how long they retain the capacity for successful fertilization.

Early Development

Methods

Embryological material was obtained during collection of swarming animals in November, 1960, when a male and a female were placed together in a jar. Although mortality due to polyspermy was high, the large number of eggs fertilized yielded an abundant supply of normally developing embryos. As soon as they were brought to the laboratory, the eggs were distributed into fingerbowls and washed five or six times. Thereafter the water in these bowls was changed every two hours, until swimming larvae appeared. Throughout the observations the cultures were kept in containers placed in a tray of running water to minimize temperature changes, and all water added to the cultures was first filtered through several layers of gravel and sand. Aeration was supplied by a small pump.

Several different methods were tried to maintain the larvae after swimming stages appeared. Some of the larvae were placed into cages made of small polyethylene containers of the type used to refrigerate food. The sides and covers of the containers were cut out to make large windows which were then covered with bolting silk, using a soldering gun with a smoothing tip to seal the material to the plastic. Four large corks attached to the corners floated the half-submerged cages in a large tank of running water. Unfortunately, larvae were able to escape through No. 18 bolting silk, and No. 20 became clogged so rapidly that within two days a microbial growth had flourished at the expense of the cultures. The same difficulty was encountered in using a current rotor device patterned after the apparatus of Galtsoff and Cable (1933; also Galtsoff, 1959). In this case, the rotating cylinder designed to keep the larvae in a small aquarium with a constant change of water was covered with bolting silk, which again clogged up rapidly. Better results were obtained with larvae placed in gallon jars half full of water, which was gently agitated by air passed through porous stones. The jars were covered with cellophane and fresh water was added as necessary to replace loss through evaporation. The relative success of these cultures suggests that the plunger-jar technique may have much to offer. Attempts to feed the larvae in different jars included the addition of diatoms (predominantly *Coscinodiscus;* also various pennate forms), a fine powder of dried clams, or substratum obtained from areas over which swarming took place. Despite the frequent occurrence of larvae with a bolus of reddish-brown matter in the gut

Figure 6. Clusters of spermatocytes at the surface of the gonad, shortly before they are released into the coelom. Ehrlich's hematoxylin, eosin.

Figure 7. Two plates of spermatids free in the coelom. The larger plate is cut horizontally, the other vertically. Melander-Wingstrand's CAH.

Figure 8. Mature spermatozoa in the coelom of a swarming epitoke. The sperm in the center shows the acrosome (arrow), two elements of the middle piece and a short portion of the tail. Melander-Wingstrand's CAH.

Figure 9. Section through a fertilized egg in anaphase of the second polar division, showing the sperm head (arrow) penetrating into the center of the egg. Ehrlich's hematoxylin.

cavity, development ceased after the sixth day, and all the larvae died by the seventeenth day.

Rate of development

The observed rate of development at a water temperature of 12.5–14° C. is shown in Table I. Calculations are based from the time the swarming male and female were placed together; since both were shedding freely, it is assumed that fertilization occurred almost immediately. The times reported by Klawe and Dickie (1957) differ considerably from the present findings. This disparity arises mainly from the very slow rate of initial cleavages indicated in their report, and decreases in the more advanced stages. Thus the period from the 4-cell stage to the swimming embryo is about 12 hours in their report, and about 9½ hours in the present schedule, whereas the interval between swimming embryo and prototroch formation is approximately 10 hours in both cases. A difference in cleavage rate, however, is not unduly surprising, since Klawe and Dickie worked with a more northern population of bloodworms and made their observations over

TABLE I

Rate of early development at 12.5–14° C.

Stage	Hours after fertilization
Polar divisions	<2
First division spindle	2½
Two-cell	3
Four-cell	4½
Eight-cell	5½
Swimming embryo	14
Trochophore	25

a wider temperature range (12–20° C.), using eggs shed and fertilized in the laboratory.

Fertilization and early cleavage

Germinal vesicle breakdown does not normally occur until fertilization. The nuclear membrane of unfertilized eggs disappears after about 10 hours at room temperature, but this is a degenerative phenomenon followed by gradual deterioration of the eggs. Within two hours of fertilization, the oocyte rounds up to a diameter of about 100 μ, and the vitelline membrane lifts away from the cytoplasm to form a wrinkled fertilization membrane 1.8 μ thick. Evidently a block to polyspermy is established by this stage, and numerous adherent sperm are lifted by the rising membrane. No external jelly layer is formed. The cortical granules of the unfertilized ovum have disappeared by this stage, but whether they contribute to the formation of the fertilization membrane is unknown.

Both polar divisions take place while the sperm head is approaching the center of the egg (Fig. 9). One of the polocytes, and occasionally the second also, divides again. Male and female pronuclei fuse in the center of the egg, and the first division spindle is completed by 2½ hours after fertilization. The chromosomes remain vesicular throughout the first cleavage and fuse to form a

lobulated nucleus in each blastomere. The nuclei round out and elongate as the centrosomes migrate in preparation for the second division, during which the chromosomes regain their basophilia and typical form. Cleavage is spiral. The first four blastomeres appear to be equal, and the micromeres of the eight-cell stage are but slightly smaller than the macromeres.

Pelagic larvae

Thirteen hours after fertilization, perfectly spherical embryos are found rotating at the bottom of the dish and soon swim up to the surface. Gastrulation, apparently by epiboly, is in progress within the following three or four hours. Young trochophores are about 115 μ in diameter and have a broad prototroch as well as a patch of long apical cilia. They show no definite reaction to light, but are strongly geonegative, concentrating at the very surface of the water. This behavior does not persist, and larvae three days old are evenly distributed throughout the upper layers of the water.

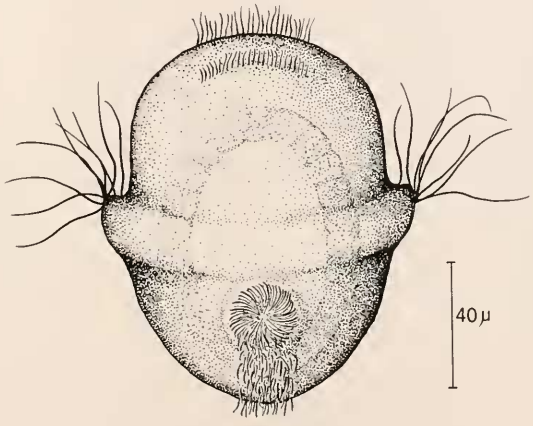


FIGURE 10. Ventral view of a six-day-old trochophore, drawn from a live specimen. Only the lateral portions of the prototroch are shown.

The six-day-old trochophore (Fig. 10) is about 120 μ long. In the legends to their illustrations of larvae, Klawe and Dickie give a length of 1.3 mm., but surely this must be a misplaced decimal point. At this stage, the larva has a complete equatorial ridge bearing two rows of well developed prototrochal cilia. An akrotroch is located ventrally, between the prototroch and the patch of short apical cilia. On the lower hemisphere, a band of cilia (neurotroch?) passes from the mouth to the posterior surface. The stomodeal opening is strongly ciliated, as is the whole surface of the gastric cavity, which seems to have in addition a ventral tract of exceptionally long cilia. When a food bolus is present, it is usually found in the anterior portion of the gut cavity, rotating counter-clockwise, as seen in dorsal view under the microscope. The gastric epithelium consists of large cells, many with vacuoles and granular inclusions. A distinct anus could not be found. The larva is not pigmented, nor does it possess eyespots.

Except for the lack of pigmentation, these larvae generally resemble the

trochophores of *G. convoluta* described by Fuchs (1911). From Madras, Aiyar (1933) reports planktonic larvae which, on the basis of their similarity to the illustrations of Fuchs, he refers to the genus *Glycera*. Judging from Aiyar's figures, however, this similarity is not especially pronounced, and the presence of eyespots in the Madras larvae makes this generic classification questionable. Aiyar also describes (as *Eone*) nectochaetes of the goniadid *Glycinde*, in which eyes are present, and possibly his trochophores should be referred to this group instead of *Glycera*. Pelagic larvae of *G. alba* from Denmark have been described by Thorson (1946), but the trochophore is 400–450 μ long, has rudimentary anal cirri, and thus appears to be a later stage than any of the other trochophores reported. Thorson's record of metatrochophores and nectochaetes is the only one known for older larvae of *Glycera*. Treadwell (1936, p. 55) reports "two very young specimens of this genus, too immature for identification" taken in a plankton net at 800 fathoms off Bermuda, but gives no description. The specimens must have been relatively large, since the nets used were of No. 2 bolting silk (Beebe, introduction to Wailes, 1936).

Attempts made at Solomons to collect older larvae and post-larval stages were unsuccessful. A No. 20 net was used to take plankton hauls, both vertical and horizontal, at several different locations during the two months following swarming, but the few polychaete larvae obtained could readily be referred to *Polydora*. Examination of bottom samples produced no better results; all of these samples, however, were from very shallow areas, and cannot be considered truly representative. The smallest bloodworms found during this study were two specimens, 3 and 4 cm. long (fixed), collected in July, 1960. Klawe and Dickie (1957) report 3 cm. as the minimum for their observations, noting that these small worms were common in May and June. They also were unsuccessful in finding pelagic larvae or newly settled juveniles, and are inclined to think that the larvae spend only a short time in the plankton.

Much of this investigation was conducted at the Chesapeake Biological Laboratory of the Natural Resources Institute, University of Maryland, and I wish to express my thanks for the generous assistance rendered by members of the Laboratory staff.

Summary

1. Information on gametogenesis in the bloodworm has been obtained by histological examination of material collected at Solomons, Maryland, in 1960 and 1961. The paired gonads begin in segments 45–47 and continue to about segment 110. They arise ventro-laterally, as retroperitoneal outgrowths near the opening of each parapodial cavity into the general coelom. Young oocytes and spermatocytes, the latter grouped in small clusters, are released from the gonads and mature in the coelomic fluid. Ripe oocytes are colorless, discoidal and about 140 μ in diameter; sperm are of the primitive type.

2. Insemination is followed by germinal vesicle breakdown, elevation of the fertilization membrane and both polar divisions. Pelagic stages appear within 14–20 hours and in a few days develop into unspecialized, apparently planktotrophic larvae. The further developmental process, including the period of metamorphosis and settling, remains completely unknown.

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